NOBLE, G. K. 1931. The biology of the Amphibia. McGraw-Hill, New York.
———, AND M. K. BRADY. 1933. Observations on the life history of the marbled salamander, *Ambystoma opacum* Gravenhorst. Zoologica 11: 89–132.
ORGAN, J. A. 1961. Studies of the local distribution, life history, and population dynamics of the salamander genus *Desmognathus* in Virginia. Ecol. Monogr. 31:189–220.
SALTHE, S. N., AND J. S. MECHAM. 1974. Reproductive and courtship patterns, p. 310–521. *In*: Biology of the Amphibia. Vol. II. B. Lofts (ed.). Academic Press. New York.
SMITH, C. C., AND S. D. FRETWELL. 1974. The optimal balance between size and number of offspring. Amer. Natur. 108:499–506.
TILLEY, S. G. 1972. Aspects of parental care and embryonic development in *Desmognathus ochrophaeus*. Copeia 1972:532–540.
TRIVERS, R. L. 1972. Parental investment and sexual selection, p. 136–179. *In*: Sexual selection and the descent of man. B. Campbell (ed.). Aldine Publ. Co. Chicago.
———. 1974. Parent-offspring conflict. Amer. Zool. 14:249–264.
WILLIAMS, G. C. 1966a. Adaptation and natural selection. Princeton U. Press. Princeton, New Jersey.
———. 1966b. Natural selection, the costs of reproduction, and a refinement of Lack's principle. Amer. Natur. 100:687–690.
WILSON, E. O. 1975. Sociobiology. Belknap Press of Harvard University Press, Cambridge, Massachusetts.

DEPARTMENT OF BIOLOGY, BROOKLYN COLLEGE OF THE CITY UNIVERSITY OF NEW YORK, BROOKLYN, NEW YORK 11210 AND DEPARTMENT OF ZOOLOGY, UNIVERSITY OF FLORIDA, GAINESVILLE, FLORIDA 32611. Accepted 26 April 1977.

Reproductive Behavior of the Anemonefish *Amphiprion melanopus* on Guam

ROBERT M. ROSS

Nineteen breeding pairs of the anemonefish *Amphiprion melanopus* and their nests were observed continuously on Guam for approximately one year. *A. melanopus* are monogamous and apparently protandrous hermaphrodites. Nest preparation consists of both anemone biting and substrate biting. Spawning occurs 2–3 hours after sunrise and lasts 1.5 hours. Eggs are mouthed and fanned by the male during the incubation period, but there is no nocturnal egg care. Hatching occurs 1.5 hours after sunset on the seventh or eighth day of incubation. Spawning activity peaks near the first and third quarters of the lunar cycle. Consequently, hatching activity peaks near full moon and new moon at the time of day when high spring tides also occur. Nocturnal hatching on high spring tides is interpreted as a mechanism for reducing predation on hatchlings due to the inactivity of some planktivores at night and the more effective dispersal of hatchlings away from the nest during high tides. There is no seasonal periodicity in spawning activity. An average of 2 spawns per lunar month per pair was observed. Estimated annual fecundity is 7,200 eggs per year for stable pairs.

RECENT studies of anemonefish (*Amphiprion* and *Premnas*) have focused on reproductive behavior and the influence of ecological parameters on the evolution of behavior (Fricke, 1974; Fricke, 1976; Moyer and Bell, 1976). Fricke's (1976) discovery of the occurrence of protandrous hermaphroditism in *A. bicinctus* reflects the importance of ecological restrictions in the case of anemonefish. Fricke predicts that sex reversal is a universal adaptation of anemonefish. In this paper I describe the reproductive behavior of *A. melanopus* and compare it to other species of anemonefish. I also discuss the lunar periodicity of spawning in *A. melanopus* as well as possible selective advantages for it.

METHODS

Between December 1974 and June 1976 I ob-

TABLE 1. SUMMARY OF NEST-ASSOCIATED BEHAVIOR IN ANEMONEFISH *Amphiprion melanopus*.

	Time of Occurrence			
	Prior to Spawning	During Spawning	During Incubation	No. of Observations
Anemone biting or tentacle nibbling	X	X		Many
Substrate biting	X		X	Many
Mutual substrate biting	X			3
Head shaking/substrate biting	X		X	5
Skimming		X		Many
Pectoral fluttering		X		Many
Egg mouthing		X	X	6
Mutual mouth touching			X	1
Mutual head shaking			X	1
Mutual head shaking/substrate biting			X	3
Egg fanning			X	Many

served 19 breeding pairs of *A. melanopus* in association with scattered aggregations of the anemone *Physobrachia douglasi* on the shallow reef top (primarily the inner reef-flat zone) at Guam, Mariana Islands (13′23″N and 144′45″ W). Groups of anemonefish at each anemone cluster are hereafter referred to as "colonies."

Determination of sex reversal was based on observations of behavior and gross anatomy. I estimated the date of spawning (with an error of ± 1 day) of newly discovered egg clutches from changes in the appearance of the eggs during embryonic development (Ross, 1976). Observations of spawning itself or evidence thereof (such as contracted anemones around nest) gave the date of spawning with no error. Each nest was checked for eggs at least once a week to insure that all spawnings of each pair were sampled. I counted the number of discrete egg-fanning sessions (typically 1–16 successive pectoral strokes preceded and followed by a pause) by seven adult males in 5 min at various times of day.

Water height analyses are based on predictions published by the U.S. Department of Commerce (1975 and 1976) for Apra Harbor, Guam. Field studies (1975) showed that both the magnitude and time of occurrence of observed high and low tides at Tumon Bay (the primary study site) were essentially the same as those predicted for Apra Harbor. Predicted water heights for given times of day are linear interpolations of the tide table, which introduces a small error due to the non-linear rate of change of water height. Comparison of measured water heights to interpolated water heights over half of a tidal cycle (0840–1437 on 22 September 1975) showed that this error did not exceed 12% of the linearly interpolated value.

RESULTS

Anemonefish colonies consisted of the adult pair and usually one or more juveniles. Female *A. melanopus* were always larger than their male partners. No polygamy was observed. Protandrous sex reversal occurred once following loss of the female from a colony. The largest juvenile became the new breeding male after a short period of rapid growth. Migration of adult fish from one colony to another was never observed.

Anemone tentacles normally covered the nests of *A. melanopus*. As much as a day prior to spawning both anemone biting (resulting in exposure of the nest) and substrate biting were observed frequently at the nest (Table 1). Spawning (eight observations of five different pairs) was always initiated between 0800 and 0900, 2–3 hours after sunrise, and lasted 1.5 hours. During spawning the female and male alternately passed over the nest (skimming) fluttering their pectoral fins rapidly. Clutches contained 200–400 eggs. During the incubation period the male fanned the eggs but only in the daytime (Fig. 1).

Eggs hatched in 7.5 or 8.5 days between 1 and 2 hours after sunset. Peak hatching occurred 1.5 hours after sunset with the aid of vigorous fanning by the male. Numerous apogonid fishes were active near nests of *A. melanopus* at night. High tides and relatively strong water currents were generally observed at the time of hatching.

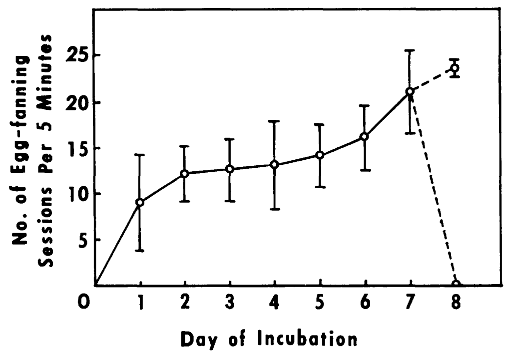

Fig. 1. Mean egg-fanning activity of male *Amphiprion melanopus* on successive days of incubation. There are four to nine observations for each day of incubation. Dashed lines indicate activity on Day 8 when either eggs were present (upper, $n = 2$) or not present (lower, $n = 4$). Vertical lines indicate $\pm$ 1 standard deviation.

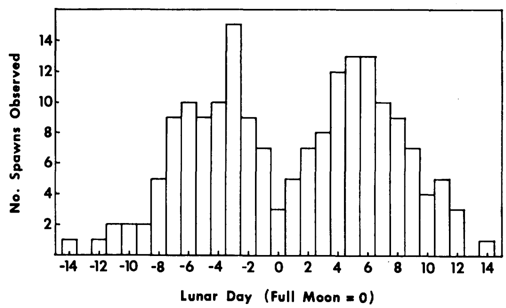

Fig. 2. Distribution of spawning in breeding colonies of *Amphiprion melanopus*.

Hatched larvae were swept away from the nest by these currents.

The reproductive cycle was related to moon phase (Table 2, Fig. 2). Two spawning peaks coincide approximately with the first and third quarters of the moon (Fig. 2). These peaks do not reflect peaks in sampling intensity. Pairs often spawned twice in a single lunar month. There was no seasonal change in spawning activity. The five pairs that were observed continuously for a year spawned nearly twice a month.

TABLE 2. PAIR FECUNDITY IN THE ANEMONEFISH *Amphiprion melanopus*.

Breeding Pair	No. Continuous Lunar Months Observed	No. Spawns Observed	No. Spawns Per Lunar Month $\bar{x}$	No. Spawns Per Lunar Month Range
1	13.5	23	1.7	1–3
2	8.3	14	1.7	1–2
3	5.0	7	1.4	1–2
4	4.5	4	0.9	0–1
5	6.9	12	1.7	1–2
6	12.2	19	1.6	1–2
7	11.8	21	1.8	0–3
8	10.0	19	1.9	2–2
9	11.0	17	1.6	1–2
10	5.7	11	1.9	1–2
11	2.3	5	2.2	2–2
12	2.0	3	1.5	1–2
13	4.3	7	1.6	1–2
14	8.5	12	1.4	1–2
15	2.0	3	1.5	1–2
16	0.9	1	–	–
17	0.9	2	–	–
18	0.3	1	–	–
19	0.3	1	–	–
TOTAL	110.4	182	1.6	0–3

DISCUSSION

A number of observations suggest that protandrous sex reversal is selectively advantageous for *A. melanopus*. First, the fish must occupy a very limited portion of the reef. Each anemone cluster, furthermore, supports only a small number of fish. Second, adult fish do not migrate from one cluster to another. Predation on anemonefish away from the anemone is high (Eibl-Eibesfeldt, 1960; Fricke, 1976), and those fish occupying an anemone cluster vigorously defend their territory against conspecific intruders (Ross, in press). Finally, reproducing females are always larger than their partners (Allen, 1972; Fricke, 1974; Moyer and Bell, 1976). These observations are consistent with the size-advantage model of hermaphroditism (Ghiselin, 1969) and with the hypothesis that female fecundity increases with age in protandrous hermaphrodites (Warner, 1975). Protandry has also been observed in *A. akallopisos* and *A. bicinctus* (Fricke, 1976) as well as in *A. frenatus* and *A. clarkii* (J. T. Moyer, pers. comm.).

The details of courtship, nest preparation, spawning and egg care in *A. melanopus* are similar to those of *A. bicinctus* and *A. clarkii*. A notable exception is the time of spawning, which was always initiated 2–3 hours after sunrise in *A. melanopus*, as opposed to almost any time of the day in other species. Also the length of the incubation period of *A. melanopus* showed much less variation (7.5 or 8.5 days)

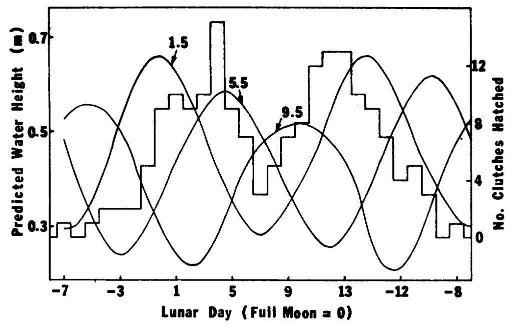

Fig. 3. Hatching frequency distribution of *Amphiprion melanopus* superimposed on predicted water height 1.5, 5.5 and 9.5 hours after sunset for equivalent lunar days. Each curve represents the mean of 12 curves (one for each lunar month in 1975) depicting water height in meters above mean lower low water.

than that of other species. Bell (1976) demonstrated that the length of the incubation period is a function of ambient temperature in *A. clarkii.* Male *A. melanopus* spend large amounts of time and energy in fanning eggs. Such a sharp division of labor probably allows for greater egg production in females than would otherwise be the case.

A runs test (Sokal and Rohlf, 1969) shows that the spawning distribution is non-random ($P \leqslant 0.01$, $n = 25$, $r = 10$, and $t_s = -3.12$). Since spawning activity peaked at approximately the first and third quarters of the moon, hatching activity peaked near full moon and new moon (due to the 7.5- or 8.5-day incubation period). Furthermore, peak hatching activity occurred 1.5 hours after sunset on the day of hatching. Tidal phenomena were analyzed in an attempt to explain these observations.

Spring tides, which produce the highest high tides and the lowest low tides, also occur at full moon and new moon. At Guam high spring tides occurred twice a day, shortly after sunrise and shortly after sunset. The eggs hatch at both the time of month and time of day when the highest waters are encountered on the reef flat (Fig. 3). It appears, therefore, that both spawning and hatching in *A. melanopus* are cyclic phenomena, evolved to take advantage of predictably occurring high tides.

The adaptive significance of nocturnal hatching on high tides may be to reduce predation on the newly hatched planktonic larvae. Though the relative percentage of planktivorous fishes active on the reef is probably higher at night than during the day, nocturnal planktivores, such as holocentrids and priacanthids, feed on larger organisms than do diurnal planktivores, such as pomacentrids and chaetodontids (Hiatt and Strasburg, 1960; Hobson, 1974). Apogonids, however, which were observed frequently near nests of *A. melanopus* in this study, are nocturnal planktivores that apparently do feed on fish larvae. Hatching on high tides may reduce such predation because of the greater capacity for escape and dispersal in a high water column. In addition, the stronger currents observed on the reef flats during high tides may sweep the hatchlings away from the nest and off the reef flat more effectively than at any other time.

The lack of seasonal changes in breeding activity in *A. melanopus* is consistent with the findings of Allen (1972) for several species of *Amphiprion* in the tropical waters of Eniwetok Atoll. At Miyake Island, Japan, *A. clarkii* were highly seasonal and spawned only six to eight times from May to October for an annual fecundity of 8,000–17,500 eggs per pair per year (Bell, 1976). I estimate an annual fecundity of 7,200 eggs per year (24 clutches) for stable pairs of *A. melanopus.*

ACKNOWLEDGMENTS

This paper is based on a thesis presented in partial fulfillment of the requirements for the MS degree at the University of Guam Marine Laboratory. I thank those associated with the Graduate Program in Biology, particularly S. S. Amesbury, J. A. Marsh, Jr., L. Raulerson and G. Boughton, for their guidance, helpful criticism and assistance. T. Tansy provided photographic documentation. The comments of J. T. Moyer, Tanaka Memorial Biological Station, Japan, and R. C. May, Hawaii Institute of Marine Biology, are appreciated. Fellow graduate students G. Ludwig and G. Heslinga also took time to read the manuscript.

Contribution No. 104 from the University of Guam Marine Laboratory.

LITERATURE CITED

ALLEN, G. R. 1972. The anemonefishes: their classification and biology. T. F. H. Publications, Neptune City, N.J.

BELL, L. J. 1976. Notes on the nesting success and fecundity of the anemonefish *Amphiprion clarkii* at Miyake-jima, Japan. Japan. J. Ichthyol. 22: 207–211.

EIBL-EIBESFELDT, I. 1960. Beobachtungen und Versuche an Anemonenfischen (*Amphiprion*) der

Maldiven und der Nicobaren. Z. Tierpsychol. 17: 1–10.

FRICKE, H. W. 1974. Oko-Ethologie des monogamen Anemonenfisches *Amphiprion bicinctus* (Freiwasseruntersuchung aus dem Roten Meer). Z. Tierpsychol. 36:429–512.

———. 1976. Bericht aus dem Riff. Piper Verlag, Munich, Germany.

GHISELIN, M. T. 1969. The evolution of hermaphroditism among animals. Quart. Rev. Biol. 44: 189–208.

HIATT, R. W., AND D. W. STRASBURG. 1960. Ecological relationships of the fish fauna on coral reefs of the Marshall Islands. Ecol. Monogr. 30: 65–127.

HOBSON, E. S. 1974. Feeding relationships of teleostean fishes on coral reefs in Kona, Hawaii. Fishery Bull. 74:915–1030.

MOYER, J. T., AND L. J. BELL. 1976. Reproductive behavior of the anemonefish *Amphiprion clarkii* at Miyake-jima, Japan. Japan. J. Ichthyol. 23: 23–32.

ROSS, R. M. 1976. Reproductive and territorial behavior of the anemonefish *Amphiprion melanopus* Bleeker. Unpubl. M.S. Thesis, University of Guam.

———. In press. Territorial behavior and ecology of the anemonefish *Amphiprion melanopus* on Guam. Z. Tierpsychol.

SOKAL, R. R., AND F. J. ROHLF. 1969. Biometry. W. H. Freeman and Co., San Francisco.

U.S. DEPARTMENT OF COMMERCE. 1975. Tide tables: high and low water predictions 1975: central and western Pacific Ocean and Indian Ocean. U.S. Government Printing Office, Washington, D.C.

———. 1976. Tide tables: *Ibid.*

WARNER, R. R. 1975. The adaptive significance of sequential hermaphroditism in animals. Amer. Nat. 109:61–82.

THE MARINE LABORATORY, UNIVERSITY OF GUAM, BOX EK, AGANA, GUAM 96910. PRESENT ADDRESS: DEPARTMENT OF ZOOLOGY, UNIVERSITY OF HAWAII, HONOLULU, HAWAII 96822. Accepted 8 April 1977.

Literature Cited

The Evolution of Hermaphroditism Among Animals
Michael T. Ghiselin
The Quarterly Review of Biology, Vol. 44, No. 2. (Jun., 1969), pp. 189-208.
Stable URL:
http://links.jstor.org/sici?sici=0033-5770%28196906%2944%3A2%3C189%3ATEOHAA%3E2.0.CO%3B2-R

Ecological Relationships of the Fish Fauna on Coral Reefs of the Marshall Islands
Robert W. Hiatt; Donald W. Strasburg
Ecological Monographs, Vol. 30, No. 1. (Jan., 1960), pp. 65-127.
Stable URL:
http://links.jstor.org/sici?sici=0012-9615%28196001%2930%3A1%3C65%3AEROTFF%3E2.0.CO%3B2-N

The Adaptive Significance of Sequential Hermaphroditism in Animals
Robert R. Warner
The American Naturalist, Vol. 109, No. 965. (Jan. - Feb., 1975), pp. 61-82.
Stable URL:
http://links.jstor.org/sici?sici=0003-0147%28197501%2F02%29109%3A965%3C61%3ATASOSH%3E2.0.CO%3B2-O